THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF VETERINARY AND BIOMEDICAL SCIENCES

THE EFFECT OF SELENIUM ON ULCERATIVE COLITIS PROGRESSION

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ABSTRACT

Ulcerative colitis is an idiopathic autoimmune disorder which currently is treated primarily with corticosteroids and other immunosuppressants. These treatments leave ulcerative colitis patients at risk for opportunistic infections and cancer. It has been shown that selenium has anti-inflammatory properties due to its ability to modulate the immune response without inhibiting it. However, no in vivo experiments have been conducted to evaluate selenium’s efficacy in treating ulcerative colitis in humans or in laboratory animals. Therefore, a preliminary selenium supplementation study was conducted in C57Bl/6 mice to evaluate the effect of supplemental selenium in treating symptoms similar to those noted in ulcerative colitis among humans. The results of this preliminary study suggested promise for selenium in reducing oxidative stress and inhibiting prostaglandin-mediated inflammation associated with ulcerative colitis. In addition, mice treated with selenium had reduced progression of this disease based on qualitative assessment of individual colons. Larger scale replications studies in laboratory animals are warranted to further clarify the efficacy of selenium for treating those symptoms. The current findings raise the possibility that selenium might eventually be shown to be useful for treating ulcerative colitis in future treatment trials involving humans.
# TABLE OF CONTENTS

LIST OF ABBREVIATIONS \(\text{iii-v}\)

LIST OF FIGURES AND TABLES \(\text{vi-vii}\)

ACKNOWLEDGEMENTS \(\text{viii}\)

Chapter 1 - Introduction
- Etiology of Ulcerative Colitis \(\text{1}\)
- Symptoms and Current Treatments \(\text{2}\)
- Macrophage Activation in the Innate Immunity \(\text{3}\)
- Arachidonic Acid Pathway and Inflammation \(\text{4}\)
- Anti-Inflammatory Effects of Selenium \(\text{5}\)
- Selenium’s Effect on Prostaglandins Formed in the Arachidonic Acid Pathway \(\text{6}\)
- Research Hypotheses \(\text{7}\)

Chapter 2 - Materials and Methods
- Mice \(\text{8}\)
- Diets \(\text{8}\)
- Induction of Colitis (Experiment 1) \(\text{9}\)
- Chronic Model of Colitis (Experiment 2) \(\text{9}\)
- Colitis Symptoms \(\text{10}\)
- Myeloperoxidase (MPO) Assay \(\text{11}\)
- Prostaglandin Dehydrogenase (PGDH) Activity Assay \(\text{11}\)
- Protein Analysis \(\text{12}\)
- RNA Analysis \(\text{14}\)
- Statistical Analysis \(\text{15}\)

Chapter 3 - Results
- Effect of Selenium on Body Weight \(\text{16}\)
- Effect of Selenium on Colon Length \(\text{18}\)
- Effect of Selenium on Colonic Blood Score \(\text{19}\)
- Effect of Selenium on Myeloperoxidase Activity \(\text{20}\)
- Effect of Selenium on Prostaglandin Dehydrogenase Activity \(\text{21}\)
- Protein Analysis \(\text{22}\)
- RNA Analysis of 15 PGDH \(\text{23}\)
- RNA Analysis of TNF-\(\alpha\) \(\text{23}\)
- RNA Analysis of IL-4 \(\text{24}\)
- RNA Analysis of IFN-\(\gamma\) \(\text{25}\)
- RNA Analysis of mPGES-1 \(\text{25}\)
- RNA Analysis of HPGDS \(\text{26}\)

Chapter 4 - Discussion and Future Applications \(\text{27}\)

References \(\text{32}\)
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>IBD</td>
<td>Inflammatory bowel disease</td>
</tr>
<tr>
<td>T&lt;sub&gt;H&lt;/sub&gt; Cells</td>
<td>Helper T lymphocytes</td>
</tr>
<tr>
<td>IL-1</td>
<td>Interleukin 1</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor α</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon γ</td>
</tr>
<tr>
<td>CD8+ Cells</td>
<td>Cytotoxic T lymphocytes</td>
</tr>
<tr>
<td>M1</td>
<td>Classically-activated macrophages</td>
</tr>
<tr>
<td>M2</td>
<td>Alternatively-activated macrophages</td>
</tr>
<tr>
<td>T&lt;sub&gt;H&lt;/sub&gt;1 Cells</td>
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<tr>
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<td>Interleukin 12</td>
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<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
</tr>
<tr>
<td>RONS</td>
<td>Reactive oxygen nitrogen species</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>T&lt;sub&gt;H&lt;/sub&gt;2 Cells</td>
<td>T helper 2 lymphocytes</td>
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<tr>
<td>PLA&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>Prostaglandin</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
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<tr>
<td>HPGDS</td>
<td>Hematopoietic prostaglandin D synthase</td>
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<td>Δ&lt;sup&gt;12&lt;/sup&gt;-PGJ&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>15-deoxy-Δ&lt;sup&gt;12,14&lt;/sup&gt;-prostaglandin J&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>Abbreviation</td>
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<td>-----------</td>
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<tr>
<td>PPARγ</td>
<td>Peroxisome proliferator-activated receptor γ</td>
</tr>
<tr>
<td>IKK</td>
<td>Inhibitor of kinase B kinase</td>
</tr>
<tr>
<td>IκB</td>
<td>Inhibitor of kinase B</td>
</tr>
<tr>
<td>NFκB</td>
<td>Nuclear factor kappa B</td>
</tr>
<tr>
<td>IKKβ</td>
<td>Inhibitor of kinase B kinase β</td>
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<td>GPX</td>
<td>Glutathione peroxidase</td>
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<tr>
<td>APCs</td>
<td>Antigen-presenting cells</td>
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<td>C 57 black/6 mice</td>
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<td>ppm</td>
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<td>kDa</td>
<td>KiloDalton</td>
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<td>Myeloperoxidase</td>
</tr>
<tr>
<td>PGDH</td>
<td>Prostaglandin dehydrogenase</td>
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<td>EDTA</td>
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</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
</tr>
<tr>
<td>NAD</td>
<td>Nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>TPER</td>
<td>Tissue protein extraction reagent</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
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<tr>
<td>PAGE</td>
<td>Polyacrylamide gel electrophoresis</td>
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<td>15 PGDH</td>
<td>15-hydroxyprostaglandin dehydrogenase</td>
</tr>
<tr>
<td>COX 2</td>
<td>Cyclooxygenase 2</td>
</tr>
<tr>
<td>mPGES</td>
<td>Prostaglandin E synthase</td>
</tr>
<tr>
<td>TBST</td>
<td>Tris-buffered saline and Tween 20</td>
</tr>
<tr>
<td>TRIzol</td>
<td>Total RNA Isolation Reagent</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>dNTP</td>
<td>Deoxyribonucleotide triphosphate</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Real-time polymerase chain reaction</td>
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</table>
LIST OF FIGURES AND TABLES

Figures

Figure 1-1. Overview of the Arachidonic Acid Pathway

Figure 2-1. Time line for Experiments 1 and 2

Figure 2-2. Dissected large intestine for colonic blood scoring

Figure 3-1. Experiment 1 Average Weight Percentages

Figure 3-2. Experiment 2 Average Weight Percentages

Figure 3-3. Experiment 1 Average Colon Lengths for Mice Sacrificed on Day 9

Figure 3-4. Experiment 1 Average Colon Lengths for Mice Sacrificed on Day 12

Figure 3-5. Experiment 2 Average Colon Lengths for Mice Sacrificed on Day 23

Figure 3-6. Experiment 1 Average Colonic Blood Score for Mice Sacrificed on Day 9

Figure 3-7. Experiment 1 Average Colonic Blood Score for Mice Sacrificed on Day 12

Figure 3-8. Experiment 2 Average Colonic Blood Score for Mice Sacrificed on Day 23

Figure 3-9. Experiment 1 Average MPO Activity

Figure 3-10. Experiment 2 Average MPO Activity

Figure 3-11. Experiment 1 Average PGDH Activity

Figure 3-12. Experiment 2 Average PGDH Activity

Figure 3-13. Experiment 1 Distal Colon: Western Blot for GPX-1 and H-PGDS

Figure 3-14. Experiment 2 Distal Colon: Western Blot for 15-PGDH

Figure 3-15. Experiment 1 Real-Time PCR for 15 PGDH

Figure 3-16. Experiment 2 Real-Time PCR for 15 PGDH

Figure 3-17. Experiment 1 Real-Time PCR for TNF-α

Figure 3-18. Experiment 2 Real-Time PCR for TNF-α

Figure 3-19. Experiment 1 Real-Time PCR for IL-4
**Figure 3-20.** Experiment 2 Real-Time PCR for IL-4

**Figure 3-21.** Experiment 1 Real-Time PCR for IFN-γ

**Figure 3-22.** Experiment 2 Real-Time PCR for IFN-γ

**Figure 3-23.** Experiment 1 Real-Time PCR for mPGES

**Figure 3-24.** Experiment 1 Real-Time PCR for mPGES

**Figure 3-25.** Experiment 1 Real-Time PCR for HPGDS

**Figure 3-26.** Experiment 2 Real-Time PCR for HPGDS

**Tables**

**Table 2-1.** Synthetic Mouse Diet

**Table 2-2.** Colonic Blood Scoring System for Distal Colon
ACKNOWLEDGEMENTS

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Chapter 1

Introduction

Ulcerative colitis and Crohn’s Disease are idiopathic autoimmune disorders that collectively comprise the vast majority of Inflammatory Bowel Disease (IBD) cases. Ulcerative colitis afflicts 1.05 million people in the United States and 1.65 million people in Europe (Neuman et al., 2006). In addition, patients suffering with chronic ulcerative colitis have an 18% higher risk of developing colorectal cancer (Eaden et al., 2001). The prevalence of ulcerative colitis is highest in western society. Caucasians are at greatest risk for contracting the disease, and most people who develop this disease are between the ages of 15 and 30. Other risk factors for ulcerative colitis include genetic factors and Jewish ancestry (Lee et al., 2009).

Etiology of Ulcerative Colitis

The etiology of ulcerative colitis is currently unknown. However, immunological, genetic, and environmental factors may all play a role in the development of colitis (Neuman et al., 2006). The dysregulation of immunity plays a key role in the pathogenesis of ulcerative colitis. This disease affects the colon and rectum, which become inflamed due to loss of barrier function of the gut epithelia, resulting in innate and adaptive immune responses to intestinal bacteria. Loss of tolerance to self-antigens leads to the activation of adaptive immunity Helper T lymphocytes (T\textsubscript{H} cells), which then stimulate the production of pro-inflammatory cytokines including interleukin 1 (IL-1), tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ), as well as chemokines that attract innate immune cells to the intestinal mucosa (Neuman et al., 2006). This infiltration leads to an inflammatory state characterized by increased oxidative stress, release of proteolytic enzymes, and resulting epithelial tissue damage. Damaged intestinal epithelial tissue results in exposure to more self-antigens. These self-antigens may activate self-reactive adaptive immune
cells, resulting in recurring infiltration and chronic inflammation (Neuman et al., 2006). This effect is exacerbated by the fact that self-recognizing T_H cells may have dysfunctional apoptosis signaling, allowing them to survive and continue the inflammatory process.

However, genetics and environmental factors may also alter the immune system, which may result in ulcerative colitis. For instance, an individual’s genetics may result in immunity with less self-tolerance, decreased negative selection during maturation, and/or reduced peripheral anergy and apoptosis of self-reactive B lymphocytes and T lymphocytes in the periphery (Ince et al., 2007). This would result in increased activation of self-reactive T lymphocytes and B lymphocytes and subsequent inflammation. If this inflammation occurs in the large intestine, it would result in ulcerative colitis. Similarly, an exogenous antigen in the lumen of the intestine may trigger an immune response, especially if the barrier function of the epithelia is compromised. If this antigen also stimulates a cross-reactive response to self-antigens, sustained inflammation would occur, also resulting in ulcerative colitis (Ince et al., 2007).

Symptoms and Current Treatments

The main symptoms of ulcerative colitis during an active flare-up include rectal bleeding, anemia, diarrhea, and weight loss. However, other symptoms including abdominal pain, vomiting, and very high frequency of bowel movements have also been reported in cases of active colitis (De Dombal et al., 1968). Because ulcerative colitis is characterized by unpredictable flare-ups at random moments, symptoms from this disease can have debilitating physical and mental effects on patients, resulting in a lower quality of life (De Dombal et al., 1968).

Because this disease is so prevalent and increases the risk of colorectal cancer by 18%, several treatments have been developed to reduce the effects of this disease (Eaden et al., 2001). One of the first-line treatments for mild ulcerative colitis is the use of aminosalicylates. For
instance, sulfasalazine has been reported to effectively maintain remission of active colitis symptoms. However, once inflammatory flare-ups occur, patients are treated with corticosteroids and other potent immunosuppressants to mitigate the active inflammation (Pithadia et al., 2011). Unfortunately, this immunoinhibition also prevents the immune mechanisms of infection clearance and cancer targeting, including natural killer and cytotoxic T lymphocyte (CD8+ cells) killing of cancer cells. Because these immunosuppressants leave patients susceptible to infection and cancer, monoclonal antibodies have been developed to target specific pro-inflammatory mediators of the immune response. One treatment that has gained recent attention is adalimumab, an anti-TNF-α antibody. Because TNF-α is the primary pro-inflammatory cytokine, treatments with adalimumab have generally been effective at mitigating flare-ups and facilitating remission of symptoms. However, individual syringe treatments of adalimumab cost more than $700 per visit (Xie et al., 2009).

Macrophage Activation in the Innate Immunity

Macrophages are key players of the innate immune response and have been characterized as classically-activated (M1) or alternatively-activated (M2) phenotypes based on gene expression profiles and functional properties. M1 macrophages are activated by a pro-inflammatory cytokine, IFN-γ, which is secreted by T Helper 1 (T_H1) cells, CD8+ cells, and natural killer cells (Martinez et al., 2009). Classically-activated macrophages secrete proinflammatory cytokines, chemokines, and enzymes. In addition, they produce reactive oxygen species (ROS), which act as important regulators of inflammatory signaling pathways. These M1 cells contain the markers including TNFα, IL-1β, IL-12, and iNOS, which result in reactive oxygen nitrogen species (RONS) (such as nitric oxide (NO)), respiratory burst, and inflammation (Nelson et al., 2011). In particular, iNOS produces NO, a potent RONS involved in respiratory
burst, from L-arginine. M1 macrophages are also characterized by amplified anti-microbial
properties and increased phagocytosis. Therefore, classically-activated macrophages are powerful
mediators of inflammation (Nelson et al., 2011).

On the other hand, M2 macrophages are activated by the complement of IFNγ, IL-4. This
cytokine is released by T Helper 2 (Th2) cells and induce the M2 phenotype, which contains
increased expression of M2 markers, including Arginase 1 (Arg1). Arg1 converts L-arginine to L-
ornithine, which results in less available L-arginine that can be used by iNOS to produce NO
(Nelson et al., 2011). In addition, L-ornithine forms the precursor for collagen and polyamine
synthesis, which play pivotal roles in the wound healing responses. Thus, Arg1 effectively
removes one of the mechanisms involved in the inflammatory process. M2 macrophages are
major contributors of the resolution of inflammation and promote tissue healing and cell
proliferation in wounded sites (Nelson et al., 2011).

Arachidonic Acid Pathway and Inflammation

The arachidonic acid pathway is a central pathway of the inflammatory process in humans
and animals. Arachidonic acid is formed from phospholipids in a hydrolysis reaction catalyzed by
phospholipase A2 (PLA2). Arachidonic acid is then either converted to leukotrienes by 5-
lipoxygenase, or converted to prostaglandin (PG) H2 by COX enzymes via a hydroperoxy
intermediate, PGG2. PGG2 is then reduced by COX to PGH2, the major diverging point of the
pathway. PGH2 can be converted to prostacyclins, thromboxanes, or prostaglandins. During
inflammatory conditions, microsomal prostaglandin E synthase (mPGES-1) converts PGH2 to
PGE2, a pro-inflammatory prostaglandin. Conversely, hematopoietic prostaglandin D synthase
(HPGDS) converts PGH2 to PGD2. PGD2 naturally is dehydrated non-enzymatically to PGJ2, Δ12-
PGJ2, and 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2), which can be used as endogenous ligands of
peroxisome proliferator-activated receptor γ (PPARγ) (Vunta et al., 2007). An overview of the pathway is described below:

![Arachidonic Acid Pathway Diagram](image)

**Figure 1-1.** An overview of the arachidonic acid pathway to the production of pro-inflammatory and anti-inflammatory prostaglandins. Red Box: prostaglandin is pro-inflammatory Blue Box: prostaglandin is anti-inflammatory

### Anti-inflammatory Effects of Selenium

When selenium is absorbed into the body, it is incorporated into selenoproteins. In the form of these selenoproteins, selenium can modulate the macrophage phenotype to alternatively activated, M2, as mentioned above (Nelson et al., 2011). M2 macrophages have anti-inflammatory properties including expressed Arg1 on the surface. Because Arg1 results in decreased amounts of NO produced from L-arginine, M2 macrophages can directly decrease the amount of RONS, leading to suppression of inflammation. In addition, these selenium-induced
M2 macrophages are characterized by their ability to resolve inflammation, while promoting tissue healing and cell proliferation in wounded sites (Nelson et al., 2011). Therefore, by inducing the M2 phenotype, selenium lowers the amount of oxidative stress while also promoting resolution of inflammation.

The anti-inflammatory effect of selenium is also due to the upregulation of glutathione peroxidase (GPX) (Vunta et al., 2007), and selenium’s ability to inhibit macrophage infiltration (Vunta et al., 2008). Because ROS are associated with inflammation, increased GPX levels reduce the amount of ROS, leading to abatement of inflammation (Vunta et al., 2007).

In addition, selenium’s anti-inflammatory effect is shown by its ability to inhibit macrophage infiltration (Vunta et al., 2008). As mentioned above, M1 macrophages are important antigen-presenting cells (APCs) for the activation of T cells, and they also result in an inflammatory response. Therefore, by preventing macrophage infiltration to the tissue, T cell activation will be limited and inflammation will be reduced (Vunta et al., 2008).

Selenium’s Effect on Prostaglandins Formed in the Arachidonic Acid Pathway

IκB kinases (IKK) phosphorylate inhibitors of kinase B (IκB), resulting in IκB’s dissociation from NFκB and polyubiquitination, resulting in IκB degradation (Vunta et al., 2007). Selenium deficiency results in exacerbation of COX2. COX2 activity leads to increased levels of pro-inflammatory PGE2, while COX1 plays an important role in the production of anti-inflammatory PGD2, PGJ2, and 15d PGJ2 (Vunta et al., 2007). In the presence of selenium, the expression of COX2 is significantly lower, resulting in an HPGDS-dependent increased proportion of PGH2 being converted to PGD2 and other anti-inflammatory prostaglandins (Vunta et al., 2007). As more anti-inflammatory prostaglandins are expressed, these prostaglandins form adducts with IKKβ, resulting in inhibition of these kinases. As mentioned above, because
activated IKKβ phosphorylates IκB (resulting in IκB’s degradation), anti-inflammatory prostaglandin inhibition of IKKβ allows IκB to remain associated to NFκB. This association inhibits NFκB such that it can not activate transcription of pro-inflammatory genes. In this way, selenium decreases pro-inflammatory enzymes COX2 and iNOS and decreased pro-inflammatory cytokine TNF-α via inhibition of NFκB, resulting in decreased inflammation (Vunta et al., 2008).

The anti-inflammatory effect of selenium is also due to PPARγ-dependent suppression of the inflammatory process. As mentioned above, selenium results in increased levels of anti-inflammatory prostaglandins. In addition to binding and inhibiting IKKβ, these anti-inflammatory prostaglandins can act as ligand that bind and activate PPARγ. This activated nuclear receptor results in the downregulation of NF-κB dependent gene expression via multiple mechanisms, including squelching of NF-κB proteins (p65 and p50) in the depression of the NFκB pathway and other inflammatory processes (Glass et al., 2010).

Research Hypotheses

Given the recent literature on the inflammatory response and selenium’s anti-inflammatory effects, we examined whether ulcerative colitis would be affected by selenium supplementation in the diets of mice. Because ulcerative colitis is highly inflammatory, we hypothesized that selenium supplementation would mitigate the amount of inflammation present in this disease. In addition, because ulcerative colitis is a chronic autoimmune disease with flare-ups of severe inflammation, we also hypothesized that selenium-fed diets will protect the mice from recurrent inflammation. These hypotheses were tested after separating the mice into two groups and feeding half of them with selenium-deficient diets and the other half with selenium-supplemented diets. The effects of selenium were analyzed using macroscopic indices of colitis progression as well as microscopic parameters of inflammation and of the presence of selenium.
Chapter 2

Materials and Methods

Mice

Twenty-one C57Bl/6 male mice were purchased for use at the Pennsylvania State University. The procedures used for these mice were analyzed and approved by the Pennsylvania State University International Animal Care and Use Committee. The mice were three weeks old when they arrived in lab. Mice were kept in 6 cages with 3 or 4 mice per cage.

Diets

Gel-based diets mixes were prepared using the following:

<table>
<thead>
<tr>
<th>Table 2-1. Synthetic Mouse Diet:</th>
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<tr>
<td>Sucrose: 2171 g</td>
</tr>
<tr>
<td>Cerelose (Dextrose): 4408 g</td>
</tr>
<tr>
<td>Vitamin-free Casein: 1800 g</td>
</tr>
<tr>
<td>Arginine: 24 g</td>
</tr>
<tr>
<td>Methionine: 16 g</td>
</tr>
<tr>
<td>Glycine: 45 g</td>
</tr>
<tr>
<td>Cysteine: 20 g</td>
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<tr>
<td>CaCO$_3$: 218 g</td>
</tr>
<tr>
<td>K$_2$HPO$_4$: 39.6 g</td>
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<td>K$_2$HPO$_4$: 50.4 g</td>
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<td>NaH$_2$PO$_4$: 119 g</td>
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<td>Cellulose (Alphacel): 300 g</td>
</tr>
<tr>
<td>Choline Chloride: 40 g</td>
</tr>
<tr>
<td>Na Ascorbate (dark): 10 g</td>
</tr>
<tr>
<td>Wesson Oil: 500 g</td>
</tr>
<tr>
<td>Water Soluble Vitamin Mix*: 20 g</td>
</tr>
<tr>
<td>*Salt Mix: 238.7 g</td>
</tr>
</tbody>
</table>

*D indicates that the salt mix was the component that varied in selenium content.

Diet mixes were stored at -20°C. Diets containing 250 g of diet mix (Table 2-1) were added to 6.25 g of heated agar in 0.375 L of distilled water. Only 250 g of diet mix were used for each preparation so that new gel diets would be fresh each week for consumption. After mixing the liquefied gel, the gel was refrigerated to solidify and used as needed. Cages were checked daily to ensure adequate amounts of food and water in each cage. In addition, animals were observed for any signs of toxicity. Selenium-deficient (containing 0 ppm selenium) and selenium-supplemented (containing 1.5 ppm) diets were provided throughout the duration of each experiment.
Induction of Colitis (Experiment 1)

Mice were fed selenium-deficient and selenium-supplemented diets for 5 weeks. Dextran sodium sulfate (DSS) (3.5%, molecular weight= 40 kDa) was dissolved in sterile water. DSS inhibits proper barrier function of intestinal epithelium in the colon, resulting in the development of ulcerative colitis symptoms (Froicu et al., 2007). These symptoms include diarrhea, rectal bleeding, and qualitative indicators of morbidity (Froicu et al., 2007). The DSS was administered in each cage’s drinking water supply on day 0, and continued to be administered for the next 5 days. On day 5, DSS administration was discontinued and normal drinking water was resumed. Starting on day 0, mice were weighed daily and observed for signs of morbidity. No mice were sacrificed due to extreme morbidity or loss of 25% or more of body weight. Three mice from each group were sacrificed on day 9 and day 12.

Chronic Model of Colitis (Experiment 2)

Eleven mice were treated with selenium-deficient diets, and 10 mice were treated with selenium supplemented diets at the beginning of the experiment. Five mice treated with selenium-deficient diets and 4 mice treated with selenium-supplemented remained following day 12. These mice were used in a second experiment to evaluate selenium’s role in a chronic model of ulcerative colitis, since patients have chronic ulcerative colitis with recurrent flare-ups of symptoms. Because these mice may have had some residual inflammation from the 3.5% DSS treatment in experiment 1, they were able to recover until Day 14. On day 14, mice were treated with 5% DSS administered in their drinking water in the same manner as the experiment 1 DSS administration. Rather than using 3.5% DSS, 5% DSS was used to observe more dramatic effects of ulcerative colitis, especially the effect on body weight. A timeline of the experiments is provided below:
The colonic blood scoring system that had been described by Froicu et al. (2007) was used in these colitis experiments. The parameters of this scoring system are listed as follows:

**Table 2-2.** Colonic blood scoring system used to measure the qualitative amount of blood in the colons of mice during experiments 1 and 2.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>no blood in whole colon</td>
</tr>
<tr>
<td>1</td>
<td>blood in less than 1/3 of colon</td>
</tr>
<tr>
<td>2</td>
<td>blood in less than 2/3 of colon</td>
</tr>
<tr>
<td>3</td>
<td>blood throughout the entire length of the colon</td>
</tr>
</tbody>
</table>

Figure 2-1. Timeline of experiments 1 and 2. The term “Sac” indicates that mice were sacrificed on this day.

Colitis Symptoms

The colonic blood scoring system that had been described by Froicu et al. (2007) was used in these colitis experiments. The parameters of this scoring system are listed as follows:

Table 2-2. Colonic blood scoring system used to measure the qualitative amount of blood in the colons of mice during experiments 1 and 2.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no blood in whole colon</td>
</tr>
<tr>
<td>1</td>
<td>blood in less than 1/3 of colon</td>
</tr>
<tr>
<td>2</td>
<td>blood in less than 2/3 of colon</td>
</tr>
<tr>
<td>3</td>
<td>blood throughout the entire length of the colon</td>
</tr>
</tbody>
</table>

Figure 2-2. Dissected large intestine for colonic blood scoring. Colonic scoring was based on the amount of blood throughout the colon.
The entire length of the colon, from cecum to anus, was removed and measured. As colitis symptoms progress, the colon tends to reduce in length. Three sections of the distal colon were collected: 1 for RNA analysis, 1 for protein analysis, and an additional section stored for future analysis. In addition, blood was collected by retroorbital sinus bleeding with microcapillary tubes and stored in -80°C. The liver and the spleen were also removed and stored with the extra piece of distal colon in liquid nitrogen.

Myeloperoxidase (MPO) Assay

Colonic tissue was suspended in ice-cold 50 mM potassium phosphate buffer, pH 6.0, containing 0.5% hexadecyltrimethyl ammonium bromide. The samples were sonicated at full power for 5-10 seconds each. Sonication was repeated 4 times with 30 second cooling intervals between sonications. These samples were then allowed to stand at 4°C for 20 minutes and then centrifuged at 12,500 g for 15 minutes at 4°C. One tenth of a milliliter of supernatant and 2.9 ml of 50 mM phosphate buffer, pH 6.0, containing 0.167 mg of O-diiodinatedihydrochloride and 0.0005% (v/v) H₂O₂ were then loaded into a spectrophotometer. Absorbances were then read every 30 seconds at 460 nm for 3 minutes to measure myeloperoxidase activity. Myeloperoxidase activity was calculated as X/mg of protein. X equaled 10 x the change in absorbance per minute/volume of supernatant in the final mixture (.03 mL). After activities were calculated, bar graphs were generated for selenium-deficient and selenium-supplemented groups.

Prostaglandin Dehydrogenase (PGDH) Activity Assay

A 100 mL Tris, ethylenediaminetetraacetic acid (EDTA), dithiothreitol (DTT) buffer was prepared with 50 mM Tris, 1 M EDTA, and 2 mM DTT in Milli-Q water, pH=7.4. A 250 μL of 10 mM nicotinamide adenine dinucleotide (NAD) to 2.5 ml of buffer was made, and 90 μL of
this mixture was added to 10 μL of protein lysate. In addition, 2 μL of PGE₂ was added for a total volume of 102 μL. The 102 μL reaction mixture was added to a spectrophotometer and absorbances at 340 nm were recorded for selenium-deficient and selenium-supplemented groups.

PGDH activity was determined as follows: \[
\text{[change in optical density x volume of reaction mixture (1mL)]/extinction coefficient (.00622) x mass of protein in mg.}
\]

After activities were calculated, bar graphs were generated for selenium-deficient and selenium-supplemented groups.

**Protein Analysis**

Protein samples were flushed with phosphate-buffed saline (PBS) containing 100 U/ml of penicillin and 100 μg/ml of streptomycin. This solution was then removed and 250 μl of tissue protein extraction reagent (TPER) with protease inhibitors was added. The samples were then homogenized and centrifuged at 10,000 G at 4 °C for 10 minutes. The supernatants were collected and protein estimations were performed using a protein estimation assay with a bovine serum albumin set. A standard 96-well plate was made with 10 μL of protein estimation standards A-H (A- 2000 μg/mL, B- 1500 μg/mL, C- 1000 μg/mL, D- 750 μg/mL, E- 500 μg/mL, F- 250 μg/mL, G- 125 μg/mL, and H- 25 μg/mL). In the following wells, 3 μL of sample were added with 7 μL of Milli-Q water for a total volume of 10 μL. Then, 150 μL of Pierce 600 nm Protein Assay Reagent was added to each well, which contained either protein estimation standards or protein sample solutions. The plate was then incubated at 37 °C for 5 minutes, and absorbance values were recorded at 620 nm using a Spectra Count plate reader. These absorbance values were used to determine the concentration of protein for each well. These concentrations were applied to load appropriate amounts of protein into a polyacrylamide gel.

A 12% (%T) gel consisting of a separating gel and stacking gel was prepared with a 7 mm 10-well comb. Gels were loaded with 1 well containing 4 μL of ladder and wells with 12 μL of
samples containing specific ratios of protein to Milli-Q water (depending on concentrations from the protein estimation assay) totaling 10 μL + 2 μL of loading dye. On the outsides of the gel, whatever wells remained were loaded with 5 μL of loading dye. Using the protein estimation values, a sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) was run at 150 volts (V) for 60-70 minutes. Then, a membrane sandwich was built using 2 sponges, 4 filter pages, 1 membrane page, and the gel. Transfer buffer (1X) was added to the sandwich, which was run in a second electrophoresis at 150 V for 90 minutes.

Following the transfer, ponceau was added to the membranes in petri dishes to check for proper transfer and to label the ladder on the membrane. Then, ponceau was decanted and the membranes were washed in Milli-Q water for 2 minutes. Five percent milk preparations were made and added to the membranes for blocking on a shaker for 60 minutes. Then, a primary antibody for either 15-hydroxyprostaglandin dehydrogenase (15 PGDH), inducible nitric oxide synthase (iNOS), glutathione peroxidase (GPX), cyclooxygenase 2 (COX 2), hematopoietic prostaglandin D synthase (H-PGDS), or microsomal prostaglandin E synthase-1 (mPGES-1) was added in 5% milk to each membrane and the membranes were left overnight on a shaker. The primary antibodies were used at various dilutions, as optimized previously by the members of the Prabhu laboratory.

The following day, the primary antibodies were stored in the -20 °C freezer and 3 washes with tris-buffered saline and Tween 20 (0.1%; TBST) were performed on the membranes for 10 minutes each on a shaker. Then, the secondary antibody (1.5 μL in 4 mL of 5% milk) was added and the membranes were put on a shaker for 60 minutes. An additional 3 washes with TBST was then performed for 10 minutes each on a shaker. Then, 1 mL of each of the 2 developing solutions (stable peroxide solution and luminol enhancer solution) was added to each membrane, and the membranes were put on a shaker for an additional 5 minutes. Finally, membranes were transferred to a cassette with film and developed for different durations based on concentration of
protein. The films were then developed and analyzed for protein levels. If no recognizable bands were seen at 15 minutes exposure, an additional highly sensitive luminescence reagent called “Femto” (50 μL; Thermo Pierce) and an additional 2 milliliters of each chemiluminescent substrate were added and membranes were put on a shaker for 5 minutes. Then, the membranes were retransferred to a cassette and films were developed for protein levels.

RNA Analysis

Distal colon samples that were used for RNA analysis were submerged in 500-1000 μL of Trizol reagent (Total RNA Isolation Reagent; TRIzol, Life Technologies). Tissues were homogenized in 1 mL of TRIzol using a hand-homogenizer. The homogenate was centrifuged at 12,000 g for 10 minutes at 4 °C. The homogenized samples were then incubated at 25 °C for 5 minutes and 0.2 mL of chloroform was added to each tube. The tubes were then shaken vigorously for 15 seconds and incubated at 25°C for 2 minutes. Tubes were then centrifuged at 12,000 g at 4 °C for 15 minutes. After the centrifugation, the aqueous phase was transferred to a fresh tube and 0.5 mL of isopropyl alcohol was added to each tube to precipitate the RNA. Next, samples were incubated at 25 °C for 10 minutes and then centrifuged at 12,000 g for 10 minutes at 4 °C. The supernatant was then removed and the RNA pellet was washed once with 1 mL of 75% ethanol. The sample was vortexed and centrifuged at 7,500 g for 5 minutes at 4 °C. Once RNA was isolated, RNase-free water was added and the samples were incubated for 10 minutes at 55 °C. Then, these samples were used in spectrophotometry to determine the RNA concentration as well as protein contamination. Generally, nucleic acids absorb at 260 nm while proteins absorb at 280 nm. A low ratio (near 1) indicates that the sample was contaminated with phenol. Samples that contained appropriate ratios (1.6-1.8 of nucleic acids to proteins) were selected to make complementary DNA (cDNA). After the concentrations of RNA in the samples
were determined, the quality of RNA was checked with electrophoresis by running the samples on a 1.8% agarose gel. A master mix was prepared with 20 μL of reverse transcriptase buffer, 8 μL of deoxyribonucleotide triphosphate (dNTP), 20 μL of primer, 10 μL of reverse transcriptase and 42 μL of water, resulting in a 100 μL mixture. Ten μL of master mix were added to each tube of RNA and the samples were run. After cDNA was transcribed, a 96-well plate was prepared using Taqman probes for 15 PGDH, TNF, IL-1β, IFN-γ, mPGES-1, HPGDS, and GAPDH. For each probe, a 70 μL master mix was prepared for a 10-sample plate. Five and a half μL were added to each well of the probe’s row. cDNA solutions were also created in master mixes with ratios of 1 μL of cDNA and 3.5 μL of Milli-Q water. 4.5 μL of sample mixtures were added to each well of the sample’s column for a total volume of 10 μL. The 96-well plate was then centrifuged at 5,000 g for 5 minutes and the reaction run in an ABI7300 real time polymerase chain reaction (PCR) machine to amplify to cDNA. Results were used to calculate Ct values, and bar graphs were made for each probe.

Statistical Analysis

Statistical analysis was performed for all quantitative data using unpaired student t tests with unequal variances using Microsoft Excel software. Based on unidirectional hypotheses, P values for in vivo studies were one-tailed and considered significant at P<0.10, while p values for in vitro studies were considered significant if P<0.05. Because both experiment 1 and experiment 2 were in vivo experiments, P values less than 0.10 were considered statistically significant. Error bars in bar graphs represent the standard error of the mean from the data.
Chapter 3

Results

Effect of Selenium on Body Weight

![Graph showing average weight percentages for selenium-deficient and selenium-supplemented mice.](image)

**Figure 3-1.** Average percentages of weight for selenium-deficient and selenium-supplemented mice (experiment 1), Sac Day 9: P=0.099, 95% CI, Sac Day 12: P=0.292, 95% CI

Weights were monitored each day starting from day 0 of the first experiment. On day 9, a large increase in the weights of the selenium-supplemented mice was noticed, while the weights of selenium-deficient mice decreased as shown in Figure 3-1. When these two groups were compared at day 9, the selenium-supplemented group had significantly higher percentages of day 0 weight than the selenium-deficient group (P=0.099). Three selenium-deficient and 3 selenium-supplemented mice were sacrificed on this day for analysis. Three additional mice in each group were sacrificed on day 12, however there were no significant differences in percentage of day 0 weight between the two groups (P=0.292).
As stated in the Materials and Methods section, experiment 2 was conducted to evaluate a chronic model of ulcerative colitis, and DSS was increased from 3.5% to 5% to more clearly determine the effect of selenium on colitis progression. As seen in Figure 3-2, selenium-supplemented mice had significantly lower weights than selenium-deficient mice on day 23 of the experiments (P=0.036).

Figure 3-2. Average percentages of weight for selenium-deficient and selenium-supplemented mice in experiment 2: Note that Day 0 refers to the continuation of the first experiment at Day 14 such that Day 9 of experiment 2 was the final day of the mouse experiments (Day 23). P=0.036, 95% CI
Effect of Selenium on Colon Length

Figure 3-3. Average colon lengths of selenium-deficient and selenium-supplemented mice sacrificed on Day 9 (experiment 1), $P=0.454$, 95% CI.

Figure 3-4. Average colon lengths of selenium-deficient and selenium-supplemented mice sacrificed on Day 12 (experiment 1), $P=0.037$, 95% CI.

Figure 3-5. Average colon lengths of selenium-deficient and selenium-supplemented mice sacrificed on Day 23 (experiment 2) $P=0.408$, 95% CI.
Colon length measurements were taken on each dissection day (day 9, day 12, and day 23). As seen in Figure 3-3, there were no differences in colon lengths between the two groups during the day 9 dissection (P=0.454) or the day 23 dissection shown in Figure 3-5 (P=0.408). However, the selenium-supplemented mice had significantly longer colon lengths in the day 12 dissection shown in Figure 3-4 (P=0.037).

Effect of Selenium on Colonic Blood Score

**Figure 3-6.** Average colon blood score of selenium-deficient and selenium-supplemented mice sacrificed on Day 9 (experiment 1), P=0.113, 95% CI

**Figure 3-7.** Average colon blood score of selenium-deficient and selenium-supplemented mice sacrificed on Day 12 (experiment 1), P=0.259, 95% CI

**Figure 3-8.** Average colon blood score of selenium-deficient and selenium-supplemented mice sacrificed on Day 23 (experiment 2), P=0.024, 95% CI
The colon samples were also evaluated by the colonic blood scoring system as shown in Figure 2-2. In Figures 3-6 and 3-7, there were no significant differences in blood scores between the two groups (Figure 3-6: \(P=0.113\), Figure 3-7: \(P=0.259\)). However, because statistical significance for in-vivo experiments was set at \(P\) value < 0.10, Figure 3-6 shows a trend toward lower blood colon scores in selenium-supplemented mice. In addition, Figure 3-8 showed that selenium-supplemented mice in experiment 2 had significantly lower colonic blood scores than selenium-deficient mice (\(P=0.024\)).

Effect of Selenium on Myeloperoxidase Activity

From Figures 3-9 (\(P=0.192\)) and 3-10 (\(P=0.413\)), there were no significant differences between the 2 groups and no trends between the groups were observed.
Effect of Selenium on Prostaglandin Dehydrogenase Activity

In addition to the MPO assay, a PGDH assay was also used to evaluate if PGE2 metabolism in selenium-deficient and selenium-supplemented mice were different. Figure 3-11 showed that PGDH activity was significantly higher in selenium-supplemented mice than in selenium-deficient mice (P=0.022) in experiment 1. Figure 3-11 also showed an increased PGDH activity mean for selenium-supplemented mice, but the difference was not statistically significant (P=0.413) in experiment 2.
Protein Analysis

**Experiment 1**

<table>
<thead>
<tr>
<th>Se-deficient</th>
<th>Se-Supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 3</td>
<td>4 5 6</td>
</tr>
</tbody>
</table>

IB: GPX1  
IB: H-PGDS  
IB: GAPDH

*Figure 3-13.* Western Blots of GPX1, H-PGDS, and GAPDH from selenium-deficient and selenium-supplemented mice (experiment 1): IB-immunoblot

<table>
<thead>
<tr>
<th>Se-deficient</th>
<th>Se-Supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 3</td>
<td>4 5 6</td>
</tr>
</tbody>
</table>

IB: 15-PGDH  
IB: 15-GAPDH

*Figure 3-14.* Western Blots of 15-PGDH and GAPDH from selenium-deficient and selenium-supplemented mice (experiment 2): IB-immunoblot

As seen in Figure 3-13, all 3 selenium-supplemented mice samples contained high levels of GPX1, indicating that they had received and absorbed selenium from the diets. In addition, the H-PGDS western blot revealed increased amounts of H-PGDS in lanes 3, 4, 5, and 6.

From Figure 3-14, 15-PGDH levels were higher than in the selenium-supplemented mice 4 and 5 than in selenium-deficient mice 2 and 3. Lanes 1 and 6 did not show any observable levels of 15-PGDH.
RNA Analysis of 15 PGDH

Figure 3-15. RT-PCRs of 15 PGDH expressions from selenium-deficient and selenium-supplemented mice (experiment 1), \( P=0.372, 95\% \) CI

Expression of 15 PGDH mRNA was examined in the cDNA samples using real-time PCR. There were no statistically significant differences between selenium-deficient and selenium-supplemented groups (Figures 3-15 and 3-16).

RNA Analysis of TNF-\( \alpha \)

Figure 3-17. RT-PCRs of TNF-\( \alpha \) expressions from selenium-deficient and selenium-supplemented mice (experiment 1), \( P=0.103, 95\% \) CI

Figure 3-18. RT-PCRs of TNF-\( \alpha \) expressions from selenium-deficient and selenium-supplemented mice (experiment 2), \( P=0.333, 95\% \) CI
Expression of TNF-α mRNA was examined in the cDNA samples using real-time PCR. As seen in Figures 3-17 and 3-18, there were no statistically significant differences between selenium-deficient and selenium-supplemented groups.

RNA Analysis of IL-4

![Figure 3-19. RT-PCRs of IL-4 expressions from selenium-deficient and selenium-supplemented mice (experiment 1), P=0.226, 95% CI](image1)

![Figure 3-20. RT-PCRs of IL-4 expressions from selenium-deficient and selenium-supplemented mice (experiment 2), no statistical values could be calculated](image2)

Furthermore, expression of IL-4 was examined using real-time PCR. Figures 3-19 did not show any significant differences between selenium-deficient and selenium-supplemented groups. In Figure 3-20, only one value of IL-4 expression was obtained, due to lack of samples, therefore, no statistical values could be calculated.
RNA Analysis of IFN-γ

![Figure 3-21. RT-PCRs of IFN-γ expressions from selenium-deficient and selenium-supplemented mice (experiment 1). P=0.191, 95% CI](image1)

![Figure 3-22. RT-PCRs of IFN-γ expressions from selenium-deficient and selenium-supplemented mice (experiment 1), P=0.231, 95% CI](image2)

The expression of IFN-γ mRNA was examined using real-time PCR. As seen in Figures 3-21 and 3-22, there were no statistically significant differences between the expression of IFN-γ in selenium-deficient and selenium-supplemented groups.

RNA Analysis of mPGES-1

![Figure 3-23. RT-PCRs of mPGES-1 expressions from selenium-deficient and selenium-supplemented mice (experiment 1), P=0.260, 95% CI](image3)

![Figure 3-24. RT-PCRs of mPGES-1 expressions from selenium-deficient and selenium-supplemented mice (experiment 2), P=0.179](image4)
cDNA samples were used to quantitate the expression of mPGES-1 by real-time PCR. As noted in Figures 3-23 and 3-24, there were no statistically significant differences between selenium-deficient and selenium-supplemented groups. However, there was a trend toward increased expression of mPGES-1 in selenium-deficient mice.

### RNA Analysis of HPGDS

![Bar chart](image)

**Figure 3-25.** RT-PCRs of HPGDS expressions from selenium-deficient and selenium-supplemented mice (experiment 1), *P*=0.415, 95% CI

![Bar chart](image)

**Figure 3-26.** RT-PCRs of HPGDS expressions from selenium-deficient and selenium-supplemented mice (experiment 2), *P*=0.177, 95% CI

To examine whether selenium status impacted the expression of H-PGDS, cDNA samples were quantitated using real-time PCR. There were no statistically significant differences between selenium-deficient and selenium-supplemented groups (Figures 3-26 and 3-26).
Chapter 4

Discussion and Future Applications

The goal of this study was to evaluate selenium’s potential to reverse the inflammatory process that is associated with ulcerative colitis. The first assessment of selenium’s anti-inflammatory properties in ulcerative colitis was conducted by determining the disease index in selenium-deficient and selenium-supplemented mice. Within this index the first parameter evaluated was percent weight because as ulcerative colitis progresses, the total weight of the animal is reduced. As was demonstrated in Figure 3-1, the selenium-supplemented group had an increase in weight while the selenium-deficient group had a decrease in weight on day 9. Therefore, we decided to sacrifice the first two sets of three mice from each group on this day. As seen in Figure 3-1, the difference between the two groups was significant on day 9, indicating that the selenium-supplemented group had lower ulcerative colitis than the selenium-deficient group. In contrast to these results, on day 12 the weights of the two groups of mice were not significantly different, possibly due to the fact that by day 12 the mice may have recovered from the DSS treatments.

Surprisingly, experiment 2’s weights did not follow the same trend as the experiment 1 sacrifice day 9 weights. As shown in Figure 3-3, the selenium-supplemented mice had significantly lower weights than the selenium-deficient mice, indicating increased progression of ulcerative colitis in the selenium-supplemented group. Therefore, the overall results from these experiments with a few mice do not suggest a trend toward increased weights in selenium-supplemented mice.

The next two parameters that were evaluated in the disease index were the colon lengths and colonic blood scores. Figure 3-4 showed a significant increase in colon length in the selenium-supplemented group, indicating lower disease progression. In addition, although Figure
3-3 and 3-5 did not show any differences in colon lengths between the 2 groups, they did show slightly higher averages of selenium-supplemented colon length compared to selenium-deficient colon length. The results from these experiments demonstrate that there may be a trend toward higher colon lengths in selenium-supplemented mice.

The colonic blood score was the final disease index parameter that was evaluated for ulcerative colitis progression. In experiment 2, there was a significant reduction in the colonic blood scores of selenium-supplemented mice, indicating that these mice had lower disease progression than the selenium-deficient mice (Figure 3-8). This result contradicts the results shown in Figure 3-2, which showed significantly lower weights in the selenium-supplemented group from experiment 2. Therefore, it is inconclusive as to whether the selenium-supplemented group had elevated ulcerative colitis progression from the disease index weights and colonic blood scores. There was no significant difference between colonic blood scores in the 2 groups (Figures 3-6 and 3-7). However, there was a trend toward reduced colonic scores in the selenium-supplemented mice.

For most of the data within the disease index including the day 9 weights, colon lengths, and colonic blood scores, selenium-supplemented mice appeared to have less ulcerative colitis progression than selenium-deficient mice. However, the weights shown in Figure 3-3 suggest that the selenium-supplemented mice may actually have increased progression in experiment 2. Therefore, the overall results from the disease index were inconclusive for ulcerative colitis progression.

In addition to evaluating the disease index parameters of ulcerative colitis, activity assays for MPO and PGDH were conducted. MPO is a lysosomal protein found in the granules of neutrophils (Masoodi et al., 2011). Because neutrophil infiltration is associated with inflammation, MPO activity is an indicator of inflammation in ulcerative colitis (Masoodi et al., 2011). As seen in Figures 3-9 and 3-10, MPO activity was not significantly different between the
two groups, demonstrating that neutrophil infiltration was not altered by the presence of selenium.

While MPO is a measure of neutrophil infiltration, PGDH is an enzyme that catalyzes the oxidation of prostaglandins for their eventual excretion in the urine. PGDH prefers PGE\(_2\) (pro-inflammatory) over PGD\(_2\) (anti-inflammatory) as the substrate for oxidation. Therefore, upregulation of PGDH indicates increased oxidation and excretion of PGE\(_2\). From the PGDH activity assays that were conducted, PGDH activity was significantly increased in selenium-supplemented mice from experiment 1 (figure 3-11). In addition, the PGDH activity was also increased in selenium-supplemented mice from experiment 2, although the increase was not statistically significant. These results indicate a trend toward increased PGDH activity in selenium-supplemented mice, thereby reducing PGE\(_2\)-mediated inflammation.

Western blots were also conducted on protein samples obtained during dissections on day 9, day 12, and day 23. From Figure 3-13, GPX1, an enzyme that reduced oxidative stress and thus inflammation, was only found in the selenium-supplemented mice from experiment 1. This finding indicates that ROS and subsequent inflammation were reduced in this group and suggests some level of efficacy for selenium in reducing inflammation via GPX1.

In addition to GPX1, H-PGDS was also elevated in selenium-supplemented mice, especially in lanes 4 and 5, as shown in figure 3-13. From the illustration of the arachidonic acid pathway shown in Figure 1-1, H-PGDS converts PGH\(_2\) to PGD\(_2\), an anti-inflammatory prostaglandin. Although lane 3 showed increased H-PGDS, the overall levels of H-PGDS were higher in the selenium-supplemented mice than in the selenium-deficient mice. Therefore, western blot results from experiment 1 indicated that selenium-supplemented mice had reduced inflammation due to higher levels of GPX1 and H-PGDS.

Western blots were also conducted in experiment 2 for 15-PGDH. As shown in Figure 3-14, 15-PGDH levels were higher in selenium-supplemented mice than in selenium-deficient
mice. This finding suggests that PGE$_2$ was being oxidized and inactivated more readily in selenium-supplemented mice. This result also parallels the trend of increased PGDH activity seen in selenium-supplemented mice in experiments 1 and 2.

In addition to the protein analysis with western blots, RNA was analyzed using RT-PCR and probed with either 15 PGDH, TNF-$\alpha$, IL-4, IFN-$\gamma$, mPGES-1, or HPGDS. Figures 3-21 and 3-22 showed that there were no significant differences between IFN-$\gamma$ in the 2 groups. However, there was a trend toward higher expression of IFN-$\gamma$ in selenium-supplemented mice. This result is surprising because IFN-$\gamma$ is considered to be a pro-inflammatory cytokine. Therefore, the trend toward increased expression of IFN-$\gamma$ in selenium-supplemented mice was contradictory to the experimental hypotheses. However, although there were no significant differences between mPGES-1 in the 2 groups (Figures 3-23 and 3-24), there was a trend toward decreased mPGES expression in selenium-supplemented mice. Referring back to the arachidonic acid pathway in Figure 1-1, mPGES-1 results in the production of PGE$_2$, which is a pro-inflammatory eicosanoid. Therefore, a trend toward decreased expression of mPGES in selenium-supplemented mice may indicate less inflammation in this group. This result also correlates with the increased levels of H-PGDS shown in the western blot from Figure 3-13 because if the mPGES-1 is expressed to a lower degree in selenium-supplemented mice, PGH2 would be shunted toward the anti-inflammatory pathway, leading to the production of PGD$_2$.

No significant differences were found in expression of 15 PGDH, TNF-$\alpha$, IL-4, or H-PGDS (Figures 3-15, 3-16, 3-17, 3-18, 3-19, 3-20, 3-25, 3-26 ). In addition, there were no trends for increased or decreased expression for any of these probes. The lack of increased expression in H-PGDS was surprising, because the western blot had increased levels of this protein (Figure 3-13). Therefore, these experiments should be replicated in larger study samples for more consistent results.
The results from these experiments were less consistent with each other. For instance, the disease index indicated that the selenium-supplemented mice had less ulcerative colitis progression than the selenium-deficient mice while experiment 2’s weight results indicated that selenium actually increased the progression of this disease. In addition, there was a trend toward higher IFN-γ expression in selenium-supplemented mice, but also a trend toward lower mPGES-1 expression in selenium-supplemented mice. Therefore, validation that selenium mitigates the inflammatory effects in ulcerative colitis was not obtained.

However, selenium did show several promising results, such as elevating mouse weights in experiment 1, elevating colon lengths in experiment 1, and reducing colonic blood scores in experiment 2. Because these are parameters of ulcerative colitis progression, the current findings raise the possibility that selenium may be able to mitigate the progression of this disease. In addition, selenium was found to increase PGDH activity and increase levels of GPX1. As mentioned, PGDH catalyzes the oxidation of pro-inflammatory prostaglandins for excretion while GPX1 reduces the levels of oxidative stress, leading to less inflammation. Because selenium was shown to enhance PGDH activity and increase levels of GPX1, selenium might eventually be shown to be effective in the abatement of inflammation in ulcerative colitis.

Future studies involving mice are warranted to replicate the current findings with larger samples sizes, in order to ensure adequate statistical power. If those studies involving laboratory animals suggest efficacy for selenium in samples involving mice, then subsequent studies may also be warranted to further clarify the potential anti-inflammatory effects of selenium in humans with ulcerative colitis.
References


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EDUCATION:
The Pennsylvania State University, Schreyer Honors College
Bachelor of Science in Immunology and Infectious Diseases
Bachelor of Science in Toxicology
Dean’s List: All 7 Completed Semesters

CLINICAL EXPERIENCE:
Global Medical Brigades, San Diego Danli, Honduras
• Performed procedures (taking blood pressure, pulse, and temperature) on patients in triage
• Helped treat over 1100 patients
• Shadowed physicians as they interacted with patients

Mount Nittany Medical Center, University Park, PA
• Walked behind post-surgery patients with a wheelchair in case they fell
• Assisted physical therapists to provide rehabilitation and care for patients within the physical therapy department
• Transported patients between the physical therapy department and their rooms.

UPMC Outpatient Center, Pittsburgh, PA
• Guided patients into the pre-operational room
• Observed podiatric surgeries
• Tended to the patient’s family by keeping them up-to-date on the surgery

RESEARCH EXPERIENCE:
Immunology Research Lab, Penn State University, University Park, PA
• Conducting independent experiments to determine selenium’s role in ulcerative colitis
• Working with murine animal models using sets of approximately 20 mice
• Discussing study hypotheses and experimental results to the research group

McGowan Institute for Regenerative Medicine - Paid Internship, Pittsburgh, PA
• Researched biofuel cell’s applicability for treating diabetes
• Evaluated the biofuel cell’s ability to produce electrical current
• Presented findings at a science conference and a poster session

HONORS/AWARDS:
• Schreyer Honors College Scholar
• Member of Alpha Epsilon Delta National Health Pre-Professional Honors Society
• Member of Phi Kappa Phi Honors Society
• Recipient of the 2011 Chappie Hill Scholarship for $1500
• Selected as Standard Bearer at Commencement, representing highest academic achievement in my major

ACTIVITIES/VOLUNTEERING:
• Mission work in New Orleans and South Dakota- rebuilt homes for families in need
• THON- raised money for children who are fighting cancer
• Penn State Glee Club (7 Semesters)- toured national and international venues (including New Zealand) with all-male choir
• Wesley Student Fellowship- led Christian youth group as Treasurer and sang in praise team
• New Zealand- went skydiving and bungee jumping with friends in Glee Club